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A prospective, phase II, single-centre, cross-sectional, randomised study investigating Dehydroepiandrosterone supplementation and its Profile in Trauma: ADaPT

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A prospective, phase II, single-centre, cross-sectional, randomised study investigating Dehydroepiandrosterone supplementation and its Profile in Trauma: ADaPT

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ABSTRACT

Introduction: The improvements in short-term outcome after severe trauma achieved through early resuscitation and acute care can be offset over the following weeks by an acute systemic inflammatory response with immuneparesis leading to infection, multi-organ dysfunction/failure (MOD/MOF) and death. Serum levels of the androgen precursor dehydroepiandrosterone (DHEA) and its sulphated ester DHEAS, steroids with immune-enhancing activity, are low after traumatic injury at a time when patients are catabolic and immunosuppressed. Addressing this deficit and restoring the DHEA(S) ratio to cortisol may provide a range of physiological benefits, including immune modulatory effects.

Objective: Our primary objective is to establish a dose suitable for DHEA supplementation in patients after acute trauma to raise circulating DHEA levels to at least 15 nmol/L. Secondary objectives are to assess if DHEA supplementation has any effect on neutrophil function, metabolic and cytokine profiles and which route of administration (oral vs sub-lingual) is more effective in restoring circulating levels of DHEA, DHEAS and downstream androgens.

Methods and analysis: ADaPT is a prospective, phase II, single-centre, cross-sectional, randomised study with a planned recruitment between between April 2019 and July 2021 that will investigate DHEA supplementation and its effect on serum DHEA, DHEAS and downstream androgens in trauma. A maximum of 270 patients will receive sublingual or oral DHEA at 50, 100 or 200 mg daily over 3 days. Females aged ≥50 years with neck of femur fracture and male and female major trauma patients, aged 16-50 years with an injury severity score ≥ 16, will be recruited.

Ethics and dissemination: This protocol has been approved by a Research Ethics Committee (Reference 18/WM/0102) on 8th June 2018. Results will be disseminated via peer-reviewed publications and presented at national and international conferences.

Trial registration: This trial is registered with the European Medicines Agency (EudraCT: 2016-004250-15) and ISRCTN (12961998). It has also been adopted on the National Institute of Health Research portfolio (CPMS ID:38158).

Trial Progression: The study recruited its first patient on 2nd April 2019 and held its first data monitoring committee on 8th November 2019. As of May 2020 there were 23 enrolled patients, with both male cohorts increasing to 100 mg. All female groups remain on 50 mg DHEA.

Article Summary

Strengths and limitations of this study

►► A phase II experimental study of a food supplement in the USA, but a class C controlled drug in the EU, in an acutely injured cohort with a large unmet need for new and effective therapies in both the short and long-term recovery from injury.

►► Aims to address what dose and route of administration are required to normalise DHEA levels in a cohort that we know have low levels, and any added benefit this restoration has upon immune and inflammatory parameters.

►► The rapid turnaround time from bedside to bench and from bench to interim analysis will minimise inappropriate dosing and expenditure of public money on ineffective doses. Thus, limiting patient exposure to insufficient dosing and unnecessary specimen collection.

Keywords: steroids, geriatric trauma, major trauma, endocrine, DHEA; DHEAS; immune function

Introduction

The improvements in short-term outcome after severe trauma achieved through early resuscitation and acute care are offset over the following weeks by an acute systemic inflammatory response with immuneparesis leading to infection, multi-organ dysfunction/failure (MOF) and death[1,2]. The combination of excessive pro- and anti-inflammatory responses impair the rehabilitation phase of trauma, including wound healing, physical and emotional recovery[3]. Upregulation of glucocorticoid biosynthesis promotes a catabolic state, lasting several weeks and associated with a significant reduction in muscle mass[4].

Analysis of gender differences in trauma has shown that women, particularly those under 30 years of age, have fewer infections and a better outcome from sepsis than men[5,6]. The protective effects of oestrogens on immune cells and organ function highlight the potential role of sex hormones in modulating trauma related immune dysfunction[7]. Cellular immunity is also influenced by hormone production, and members of our group have shown that the adrenal response to stress, specifically the ratio of cortisol to dehydroepiandrosterone sulphate (DHEAS), is linked to neutrophil bactericidal function, specifically superoxide production[8,9].

Dehydroepiandrosterone (DHEA) and its sulphate ester DHEAS are the most abundant steroids in the human circulation; DHEA is a precursor of sex hormones modulating several physiologic processes including metabolism, muscle protein synthesis and cardiovascular function[10,11]. DHEA is converted to active androgens in peripheral target cells including immune cells[12] and is also converted to DHEAS by the action of DHEA sulfotransferase (SULT2A1)[13]. We have shown that DHEA sulphation is down-regulated in acute inflammation systemic inflammatory response syndrome (SIRS) and sepsis [14]. Hazeldine et al reviewed the

numerous immune effects of DHEA [15] highlighting how DHEAS, but not DHEA, enhances neutrophil superoxide generation via a protein kinase C (PKC) mediated pathway, a vital immune response in fighting bacterial infections[16].

The roles of DHEA and DHEAS in severe injury are relatively unexplored. The majority of studies focus upon cortisol responses[17], whereas our data suggest that it is the cortisol: DHEAS ratio post-trauma that has a superior prognostic ability[18,19]. Although critical for survival, prolonged hypercortisolaemia is known to be detrimental in part due to its immunosuppressive effects[20]. This intra-adrenal shift causes decreased levels of circulating testosterone and oestrogen, resulting in rapid lean body mass loss[21], in addition to increased susceptibility to infection and sepsis[22]. Correcting the cortisol: DHEA or cortisol: DHEAS ratio via the administration of DHEA has yet to be undertaken in traumatically-injured patients despite DHEA and DHEAS being below the reference ranges for 6 weeks and 6 months post-injury respectively[4].

DHEA is subject to first-pass metabolism in healthy individuals, which in turn may lead to a non-physiological metabolism after an oral dose[23]. Bypassing first-pass metabolism using a sublingual or buccal preparation[24] should improve the bioavailability of DHEA [25,26]. Previous studies in the healthy older people have shown that supplementation with 50 mg DHEA orally once daily can restore both serum DHEA and DHEAS levels to that of men and women in the third decade of life[23,27]. Moreover, the current literature suggests that DHEA supplementation may not only be beneficial for immune function but extend to bone remodelling, muscle composition, psychological and neurological improvements[28].

This study will seek to estimate the optimal dose and route of administration in trauma patients of DHEA to increase serum levels of DHEA and DHEAS to those seen in healthy adults. We also aim to find the optimal dose to enhance immune function, as demonstrated through

changes in neutrophil phagocytosis and ROS production. The pilot data generated from the study is necessary to develop the protocol for a randomised controlled trial that will determine whether DHEA supplementation may improve outcomes in injured patients.

Rationale

Justification of the patient population

Although improvements in short-term outcomes from traumatic injury via aggressive early resuscitation and acute care, over 5 million people worldwide die each year from serious injury[29]. With improved pre-hospital medicine mitigating the immediate threats to life, it is the following weeks after traumatic injury, that has seen the dysregulated SIRS in susceptible patients. The SIRS response is accompanied by a compensatory anti-inflammatory response (CARS)[30] to restore homeostasis. SIRS and CARS may progress to the persistent inflammation, immunosuppression and catabolism syndrome (PICS)[31,32]. PICS further compounds the insult from the initial injury and results in an increased risk of infection, MOF and late deaths[1,2]. Therefore, strategies to mitigate these detrimental outcomes for patients are needed in the short, medium and long-term care of trauma patients.

The young trauma cohort will be recruited alongside a cohort of older (≥50 years old) female patients who have sustained a hip fracture at the neck of femur (NOF). According to the National Hip Fracture Database (2018), the NOF burden upon the UK economy is estimated to be around £1bn per annum[33]. This considerable cost is set to rise as the population ages. By the age of 40, decreasing serum levels of DHEA and DHEAS are observed in both sexes[34,35]; a phenomenon sometimes referred to as “adrenopause”. Circulating levels of DHEA and DHEAS are lower in women than in men; however, in post-menopausal women, adrenal

androgens are a source of almost all active oestrogens[36]. To the best of our knowledge, there has been no traumatic injury or NOF interventional studies supplementing DHEA or DHEAS, despite its therapeutic potential in the immediate to longer-term care of the young and aged trauma patient. In this pilot study, we will recruit a female NOF cohort as well as a young adult trauma cohort with both male and female patients presenting at the MTC and Intensive Care Unit (ICU) at QEHB.

Choice of treatment

The DHEA doses chosen in this study (50 mg, 100 mg and 200 mg) were selected based upon *in vivo* studies that demonstrated these doses to be both safe and effective at raising the levels of DHEA to that of healthy young adult levels[37]. DHEA is at its highest in the third decade of life. After which there is a steady decrease over the life course in both males and females. These doses have also previously been used in adrenal insufficiency[38], older people[39], and young males[40] and females[24], causing transient hyperandrogenism with acne being the most frequently reported side effect[41,42]. DHEA is activated to downstream androgens by stepwise conversion catalysed by several steroidogenic enzymes. However, we do not know whether the expression and activity of these enzymes are affected by major trauma and inflammation. There is evidence that inflammation and trauma affect DHEA sulfation[4,14] and may shift the conversion of DHEA to a higher rate of androgen activation.

Trauma and inflammation can impact adversely on gut absorption [43] and hepatic first-pass metabolism [44]. Therefore, we have chosen to administer DHEA as a sublingual preparation. Sub-lingual administration is commonly used by nursing staff in the ICU context, especially in those patients who have contraindications to oral administration or by patients wishing to self-administer [45].

OBJECTIVES

Primary Objective

The primary objective of this study is to establish the daily dose of DHEA that restores serum DHEA levels to at least 15 nmol/L, i.e. the mid healthy adult reference range, in trauma and hip fracture patients after 3 days of supplementation.

Secondary Objective

Secondary objectives include observing the effect of single and multiple DHEA doses on circulating DHEA, DHEAS and downstream androgens. Additionally, we will investigate whether route of DHEA administration (oral vs sublingual) modulates the profile of circulating DHEA. This will be determined by assessing the efficacy of each route via statistical modelling. The immune response to the DHEA supplementation will be assessed as a marker of potential clinical relevance.

METHODS

Trial Design

ADaPT is a prospective, randomised, open-label, trial conducted in male and female adult trauma patients and older females who have suffered a fracture of the neck of the femur. This is a single-site study with patients recruited from Queen Elizabeth Hospital, Birmingham, UK (QEHB). Three doses of DHEA will be investigated in this trial: 50, 100, 200 mg, each administered once daily for 3 days via either oral or sublingual tablets. The trial has an adaptive design in order to answer both the primary and secondary objectives, with regular interim analysis to minimise the investigation of inactive doses. The trial consists of two components. The first component of the trial is to determine the dose of DHEA needed to sufficiently raise serum DHEA levels to at least 15 nmol/L after 3 days of DHEA administration.

Based on previous work, 15 nmol/L has been selected as this is the lower acceptable level of DHEA observed in healthy young adults. Our recent analysis of the steroid response to trauma has shown that DHEA levels were very low and often undetectable for several weeks after trauma [4]. The second component of the trial is to investigate if DHEA will enhance neutrophil function. The study was approved by The Medicines and Healthcare products Regulatory Agency (MHRA) for the use of DHEA as an investigational medicinal product. Ethical approval was obtained from the West Midlands Research Ethics Committee (Reference 18/WM/0102). The trial will be conducted in accordance with the Declaration of Helsinki (World Medical Association 2008). **Figure 1** summarises the patient pathway of the trial. The protocol (version 5.0, 28th June 2019) has been prepared in accordance with the SPIRIT guidelines [46].

Patient selection

A maximum of 270 patients will be enrolled across three patient groups (young male trauma, young female trauma and female hip fracture). These trauma patient groups have been selected due to the immuneparesis effects caused by the acute systemic inflammatory response that follows severe trauma. The hip fracture group was selected as they have low serum DHEA and DHEAS due to adrenopause, and there are several papers showing an association between HPA axis activity measures and outcomes in these patients[9,19,47,48]. Patients admitted to QEHB will all be pre-screened and assessed for eligibility. Patients will be screened between 07:00 – 20:00, 7 days a week. Potential participants will have an assessment of their medical history and current trauma injury and the eligible patients will be recruited. The eligibility criteria have been split into trauma patients and hip fracture patients (**Table 1**). The study will not exclude NOF patients with dementia where supplementation is

currently being tested in the prevention and treatment of age-related cognitive impairment without deleterious effects [49].

Table 1 Patient inclusion and exclusion criteria

Trauma patients inclusion criteria
- Aged 16 - 50 years of age
- Severely injured trauma patient (Injury severity score (ISS) ≥16 and ≤50)
- Admitted to University Hospital Birmingham within 6 days of trauma
- Anticipated to be an inpatient for the 12-day trial period
Trauma patients exclusion criteria
- Ages <16 or >51
- ISS <16 or >50
- Isolated brain injury
- Unlikely to survive the study period
- Known hormone sensitive malignancy
- Known Prostatic hypertrophy (M)
- Female patients taking HRT medication
- Intake of any drugs that interfere with adrenal function in the last 3 months:
Increased metabolism of glucocorticoids • corticosteroids
Impaired glucocorticoid action Peripheral glucocorticoid insensitivity • Glucocorticoid receptor antagonist—mifepristone. • Suppression of glucocorticoid-induced gene transcription—chlorpromazine, imipramine.
Inhibition of steroidogenic enzymes involved in cortisol production • Inhibition of mitochondrial (type 1) cytochrome P450 enzymes (CYP11A1, CYP11B1, CYP11B12)—ketoconazole, fluconazole, itraconazole, etomidate, metyrapone, aminoglutethimide. • Inhibition of 3β-HSD2—trilostane.
- Pre-existing liver impairment or chronic liver failure
- Previous or current malignancy or invasive cancer diagnosed within the past 3 years except for adequately treated basal cell and squamous cell carcinoma of the skin and in situ carcinoma of the uterine cervix
-
- Pregnant and/or breast-feeding females (women of childbearing potential to complete serum pregnancy test)
- Known hypersensitivity to the active substance or excipient
- Known thromboembolic events in the last 12 months and any predisposition to thrombosis e.g. factor V leiden
Hip fracture patients inclusion criteria
- Aged 50 years and older
- Female
- Neck of femur fracture

- Admitted to University Hospital Birmingham within 6 days of fracture
- Anticipated to be an inpatient for the 12 day trial period
Hip Fracture patients exclusion criteria
- <50 years old
- Unlikely to survive the study period
- Previous or known hormone sensitive malignancy
- Intake of any drugs that interfere with adrenal function in the last 3 months: **Increased metabolism of glucocorticoids** • Concomitant use reduces corticosteroid concentrations **Impaired glucocorticoid action** Peripheral glucocorticoid insensitivity • Glucocorticoid receptor antagonist—mifepristone. • Suppression of glucocorticoid-induced gene transcription—chlorpromazine, imipramine. **Inhibition of steroidogenic enzymes involved in cortisol production** • Inhibition of mitochondrial (type 1) cytochrome P450 enzymes (CYP11A1, CYP11B1, CYP11B12)—ketoconazole, fluconazole, itraconazole, etomidate, metyrapone, aminoglutethimide. • Inhibition of 3β-HSD2—trilostane. -
- Pre-existing liver impairment or chronic liver failure
- Previous or current malignancy or invasive cancer diagnosed within the past 3 years except for adequately treated basal cell and squamous cell carcinoma of the skin and in situ carcinoma of the uterine cervix - Pregnant and/or breastfeeding (women of childbearing potential to complete serum pregnancy test)
- Known hypersensitivity to the active substance or excipient
- Females on Hormone Replacement Therapy medication
- Known thromboembolic events in the last 12 months and any predisposition to thrombosis e.g. factor V leiden

Randomisation

Patients who meet the eligibility criteria and provide consent are randomised to receive DHEA via either oral tablets or sublingual tablets once daily for 3 days using a 1:1 randomisation ratio. With three populations of patients (male-trauma, female-trauma and female-hip fracture) and two routes of administration, there will be 6 groups in total. Randomisation will take place using a secure web-based tool. Nursing and medical staff will use the Clinical RESearch Tool (CREST), developed at University Hospitals Birmingham Foundation Trust

(UHBFT), to randomly assign patients and provide an anonymised electronic case report form, for trial management, data collection and adverse event reporting. The prevailing dose of DHEA (50, 100 or 200 mg) for administration in a group will be adapted in response to sequential analysis of interim outcomes. Each group will have its own dose. Blinding is not possible as the difference in DHEA delivery method is evident to both the clinician and the participant. If a contraindication to oral or the sublingual route present prior to commencing the study intervention, forced randomisation will occur.

Study Intervention

The supplementation of DHEA will commence in the second week after injury, which has previously been shown to be a time when trauma patients become maximally unwell as a result of sepsis and multiple organ failure[50]. Additionally, this time point has been selected to optimise patient recruitment from both the NOF cohort and trauma patients (median stay 18 days s 13 days respectively) both DHEA and DHEAS levels post-injury [51]. Recruiting an in-hospital cohort provides an opportunity to monitor patients and assess the impact that this class C controlled drug has upon the serial steroid profile and immune function during a period of vulnerability over 3 days of administration.

Participant timeline

The trial intervention will occur over three days, during the first 12 days while inpatients at QEHB. Three doses of DHEA will be investigated in all patient, and treatment will occur on day 8, 9 and 10 only. All cohorts will begin the study on 50 mg, and the dose administered to the next eligible patient to be included within a cohort will be escalated when interim analysis

determines if the primary objective has been reached. At no point will any patient escalate once they have been allocated a dose of DHEA.

Dose escalation

Dose escalation for the DHEA restoration part of the trial is dependent upon the serum DHEA levels. A dose that restores serum DHEA to ≥15 nmol/L (referred to as 'normalise DHEA') is sought in at least 13-out-of-15 patients or approximately 85% of patients. The decision to escalate dose in a cohort will be driven in the main by the outcomes of the patients in that cohort. However, valuable additional information will be garnered from the outcomes of patients in other cohorts. Flexible information sharing across related groups will be achieved using hierarchical regression models.

Once a dose has been established to normalise DHEA levels within a group and at least n = 15 have completed it, the DHEA will be escalated to the next dose to satisfy the second component of the trial; to determine whether further increases in DHEA supplementation will enhance immune function. The immune response component will focus on neutrophil phagocytosis and ROS production which will involve fewer research samples. N = 15 patients (within a cohort) will complete the immune response part of the trial at each subsequent dose escalation. If 50 mg is established to be sufficient to normalise DHEA the dose will be escalated twice to investigate whether 100 mg or 200 mg is optimal for increasing the immune response. Both 100mg and 200mg have safely been used in previous studies[52,53], but this has not been addressed in the context of trauma. Each group will be escalated independently of each other (**figure 2**).

Patient and public involvement (PPI)

Before the beginning of the study, patients and lay members of the 1000 elders group at the University of Birmingham were invited to group PPI sessions held by the Surgical Reconstruction and Microbiology Reconstruction Centre (SRMRC) at QEHB. During these interactive group sessions, discussions were undertaken to highlight the work that was planned to be undertaken to address previously highlighted problems in their recovery from traumatic injury. Members of the group informally looked at, developed and passed comment upon study design and patient paperwork- contemporaneous records were generated. Upon entry and active participation with the ADaPT study, patients were asked if they would like to become members of the PPI group and assist in the ongoing evaluation and future dissemination of the project. The participants will be asked to participate in a grant application should the results of this study be warranting a more extensive phase 3 multicentre trial.

Primary and secondary outcomes

The primary outcome for the study is serum DHEA after 3 days of DHEA supplementation. Previous research into DHEA levels and DHEA supplementation use DHEAS as the primary endpoint for determining whether the supplementation has been effective at raising levels. However, DHEAS levels do not act as a proxy marker for DHEA levels in the trauma population[51]. Utilising liquid chromatography-tandem mass spectrometry (LC-MS/MS) we have shown that DHEA and DHEAS both behave differently after trauma injury[54]. Animal models of trauma have demonstrated improvements in hyperglycaemia[55], decreased mortality after trauma-induced haemorrhage[56], neurogenesis[57] and wound reperfusion[58]. Human studies including a recent meta-analysis suggested that DHEA supplementation may

be beneficial in increasing bone mineral density (BMD)[59] in women, increasing muscle strength[60], improving mood in those with moderate depression[61] and adrenal insufficiency [38]. These potential restorative immunological, physiological and psychological benefits seen in animals and human studies can only be investigated once the appropriate dose of DHEA to restore normal levels and the most suitable administration route has been identified. We know that supplementation of DHEA in healthy subjects via oral administration will result in significant first-pass metabolism and, thus, more extensive conversion of DHEA to DHEAS than is observed via, e.g. transdermal administration [62] which is why we plan to compare oral vs sublingual DHEA administration.

One potential instantaneous benefit to trauma patients, which may be observed systemically after a dose of DHEA, is a positive effect upon the bactericidal function of neutrophils[16] by enhancing reactive oxygen species (ROS) generation via activation of NADPH oxidase[63]. Neutrophil function will therefore be investigated as a secondary outcome, with limited expectations on the results of the pilot data, given that DHEA will only be administered for 3 days.

Steroid Analysis

DHEA and downstream androgens will be quantified using a validated liquid chromatography-tandem mass spectrometry (LC-MS/MS) multi-steroid profiling method carried out on a Waters Xevo-XS, with acquity uPLC, using a water/methanol (0.1% formic acid) gradient system and a HSS T3, 1.8 µm, 1.2x50 mm column. Steroids are extracted via liquid-liquid extraction using tert-butyl methyl ether (MTBE), following the addition of an internal standard and protein precipitation using acetonitrile. The MTBE layer containing steroids was

transferred and evaporated under nitrogen then reconstituted in 125 µL of 50/50 methanol/water before analysis. Steroids will be quantified against a calibration series ranging from 0.05 to 250 ng/mL[64–68].

DHEAS was measured separately. 20 µL of serum was spiked with internal standard, followed by 100 µL of acetonitrile and 20 µL of ZnSO4. The samples were then centrifuged and 100 µL of the solution was transferred to a new vial, dried and reconstituted in 200 µL of methanol/water prior to liquid chromatography tandem mass spectrometry analysis as described by Chadwick et al[69]. DHEAS will be quantified against a calibration series ranging from 250 to 8000 ng/mL.

Neutrophil Function

Trauma initiates a "stress response" through the endocrine, metabolic and inflammatory systems. The primary endocrine response is to produce catecholamines and corticosteroids, raising the immune response and mobilisation of neutrophils[70]. Neutrophil function will be analysed through the validated PhagoBURST™ and PhagoTest™ kits (Glycotope Biotechnology GmbH, Germany) to assess superoxide generation and phagocytosis, respectively. This gives a pilot opportunity to test whether DHEA supplementation improves the immune response and in turn, has the potential to protect against infection. However, benefits might only be detectable after a period of DHEA supplementation that is substantially longer than three days.

Pro and anti-inflammatory Cytokines

Prolonged CARS may leave the recovering patient susceptible to increased risk of late infection[71]. The cytokine storm of IL-6 and IL-10 have demonstrated a strong association with

the severity of injury and mortality [72], and less so sepsis[73]. These post-injury cytokines are also known to affect the peripheral target tissues that are involved in steroid metabolism[74]. The post-injury pro and anti-inflammatory cytokines assessed have been selected based on previous work from our group[75].

Tolerance – gastric residual volume

Swallowing difficulties, facial injuries or a non-functioning gut may prohibit sublingual or oral administration and compliance to the study protocol. Therefore, an adaptable study design is needed to generate pilot data. By monitoring gastric residual volumes (GRV), a surrogate marker of gastrointestinal motility[76], we will regard a GRV persistently exceeding 250ml as intolerable.

Treatment Compliance and Evaluability

To meet study compliance and be considered evaluable, the following must be satisfied:

- Patients must be sufficiently dosed on at least one day of DHEA administration.
- If a patient fails to consume the intended IMP, or vomits within 1 hour of consuming the IMP, this dosing will be classed as *insufficient*.

Data Monitoring Committee

Data analyses will be supplied in confidence to an independent Data Monitoring Committee (DMC), which will be asked to advise on whether the accumulated data from the trial, together with the results from other relevant research, justify the continuing recruitment of further patients. The DMC will operate under a trial-specific charter based upon the template created by the Damocles Group.

Results will be provided to the DMC and discussed via teleconference at a minimum. In consultation with the trial statistician, the DMC will meet when any cohort undergoes a dose

escalation decision. Additional meetings may be called if recruitment is much faster than anticipated and the DMC may, at their discretion, request to meet more frequently or continue to meet following completion of recruitment. An emergency meeting may also be convened if a safety issue is identified. The DMC will report directly to the Trial Management Group. The DMC may consider recommending the discontinuation of the trial if the recruitment rate or data quality is unacceptable or if any issues are identified, which may compromise patient safety. The trial would also stop early if the interim analyses showed differences between treatments that were deemed to be convincing to the clinical community. Data monitoring members have undertaken their initial review of the first sixteen patients on the 8th November 2019. The outcome of this DMC required all-female cohorts to continue 50 mg of DHEA (both the sublingual and oral), with both male cohorts increasing to 100 mg.

Statistical Analysis

Sample Size

The maximum sample size will be 270 (six groups of 15 participants being administered three different dose-levels). However, we predict realised sample size to be smaller as there are likely to be early opportunities to escalate dose-level within a group. Following consultation with a trial statistician n = 15 was chosen to provide a modest amount of information on the primary outcome at each dose in each group while allowing recruitment to be completed in a reasonable amount of time. Statistical power calculation has not been performed as we are not applying a null hypothesis significance testing approach.

Primary outcome

Serum DHEA concentrations will be summarised as means and standard deviations (or medians and inter-quartile ranges, if non-normal) at each time-point and dose in each cohort. The observed rate of DHEA normalisation will be reported at each dose in each cohort with confidence intervals calculated using Wilson's method. The cohorts sample sizes are small, so these cohort-specific analyses are likely to be relatively imprecise. However, the total sample size is large for the trial phase, and there is much information in the cohort structure that will likely be pertinent to understanding the outcomes. Supplementary analyses to support dose decisions will be provided using hierarchical regression models that analyse outcomes from all cohorts and doses together while reflecting cohort memberships. These models are explained below.

Modelling serum DHEA concentrations and DHEA-normalisation probability

We propose hierarchical Bayesian models to analyse serum DHEA outcomes in all cohorts simultaneously. An intercept will be included to estimate the mean population-level response common to all cohorts plus further terms to reflect effects for dose, patient type, and administration method. Further population-level terms (also called fixed effects) will be considered, including baseline DHEA level and age. Interactions will be considered, as appropriate. Group-level terms (also called random effects) to reflect cohort and patient heterogeneity will be considered.

Modestly informative or regularising priors will be used that anticipate little or no effect (i.e. have a mean close to or equal to zero) but restrict the range of parameter values to those that are feasible (i.e. do not place undue or unrealistic probability mass in wide distribution tails). Such priors can be considered informative of scale but not location in that they do not

anticipate effects but rule out infeasible values. They are effective at dissuading models from over-fitting and aiding convergence in the posterior sampler when there are many parameters. Information criteria (e.g. WAIC or LOOIC) will be used to find parsimonious but informative models.

It is anticipated that: the probability of DHEA normalisation will be modelled using a generalised linear model with logit link function, and post-baseline DHEA will be modelled using a generalised linear model with identity or log (for positive data) link functions.

Interim Analysis

There will be an interim analysis presented when any cohort undergoes a dose-escalation decision, as previously described. The particular objective of this analysis will be to assess if the rate of DHEA normalisation is too low and whether there is evidence that motivates investigating a higher dose in that cohort. The primary and supporting analyses of the primary outcome will be presented, as described above.

Ethics and Dissemination

This protocol has been approved by a Research Ethics Committee (Reference 18/WM/0102) on 8th June 2018. Results will be disseminated via peer-reviewed publications and presented at national and international conferences. This will be coordinated with members of the research team, both past and present. The study investigator is responsible for communicating important protocol modifications to relevant parties.

Trial Progression

The study recruited its first patient on 2nd April 2019 and held its first data monitoring committee on 8th November 2019. Currently, there are 23 evaluable patients, with both male cohorts increasing to 100 mg. All female groups remain on 50 mg DHEA. The dose escalation also coincided with the first sponsor audit of ADaPT. Site audits will occur at times of escalation and interim analysis until the study is completed.

Figure Legends

Figure 1: A trial flowchart describing the patient journey in through the ADaPT study.

* Due to the nature of the injury, informed consent can be sought from a professional legal representative or personal legal representative if the patient does not have capacity. Consent from the patient will be obtained at the earliest opportunity by research team members.

**24hr bloods and consent will only be collected within 24hrs of injury. The omission of this sample collection does not render a patient unevaluable.

Figure 1: Indicative flowchart to explain dose escalation design of the ADaPT trial

* Cohorts: oral-male trauma, sublingual-male trauma, oral-female trauma, sublingual-female trauma, oral-female hip fracture and sublingual-female hip fracture.

Declarations

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Availability of data and material: Data will be made available via online repositories.

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Author contributions: CB, KB and CP have prepared the manuscript. CB CP, MAF, WA, JL, CAG, AT, LC, JH, KB, AA, DB, RC, K Young, were involved in the methodological design and drafting of the trial protocol. JL, CB, CE, KM validated laboratory equipment and sample analysis for PB and PT testing. AD undertook all aspects are pharmacy procedure and IMP negotiations. K Yakoub, ET, MAF, RC, CB enrolled participants, assisted with data collection and study-

specific procedures. LC, AT, FS, WA undertook the LC-MS validation and processing of patient sex steroids samples. KB is the trial statistician who designed and wrote the analysis plan and code for randomisation of patients and times. CP, AA, DB are the trial coordinators. All authors reviewed and edited the manuscript.

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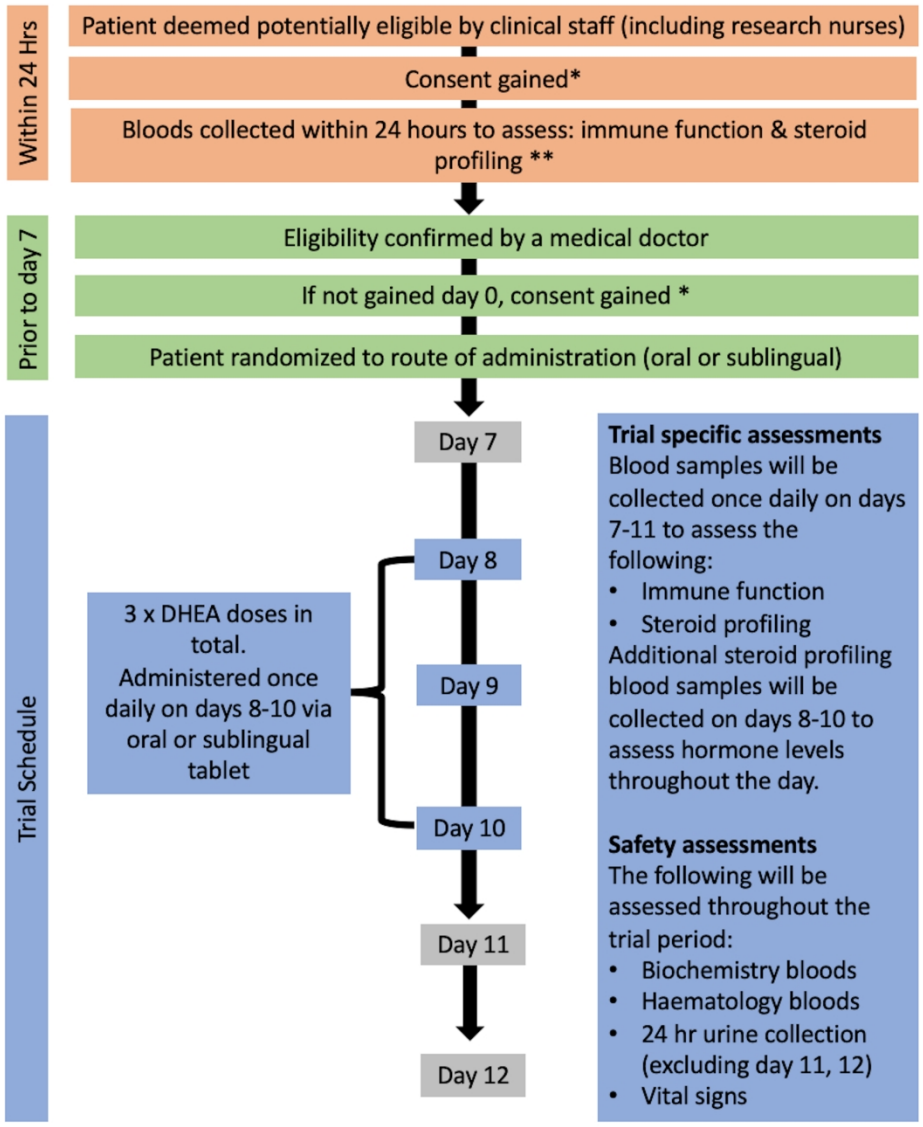

Figure 1: A trial flowchart describing the patient journey in through the ADaPT study.
* Due to the nature of the injury, informed consent can be sought from a professional legal representative or personal legal representative if the patient does not have capacity. Consent from the patient will be obtained at the earliest opportunity by research team members.
**24hr bloods and consent will only be collected within 24hrs of injury. The omission of this sample collection does not render a patient unevaluable.

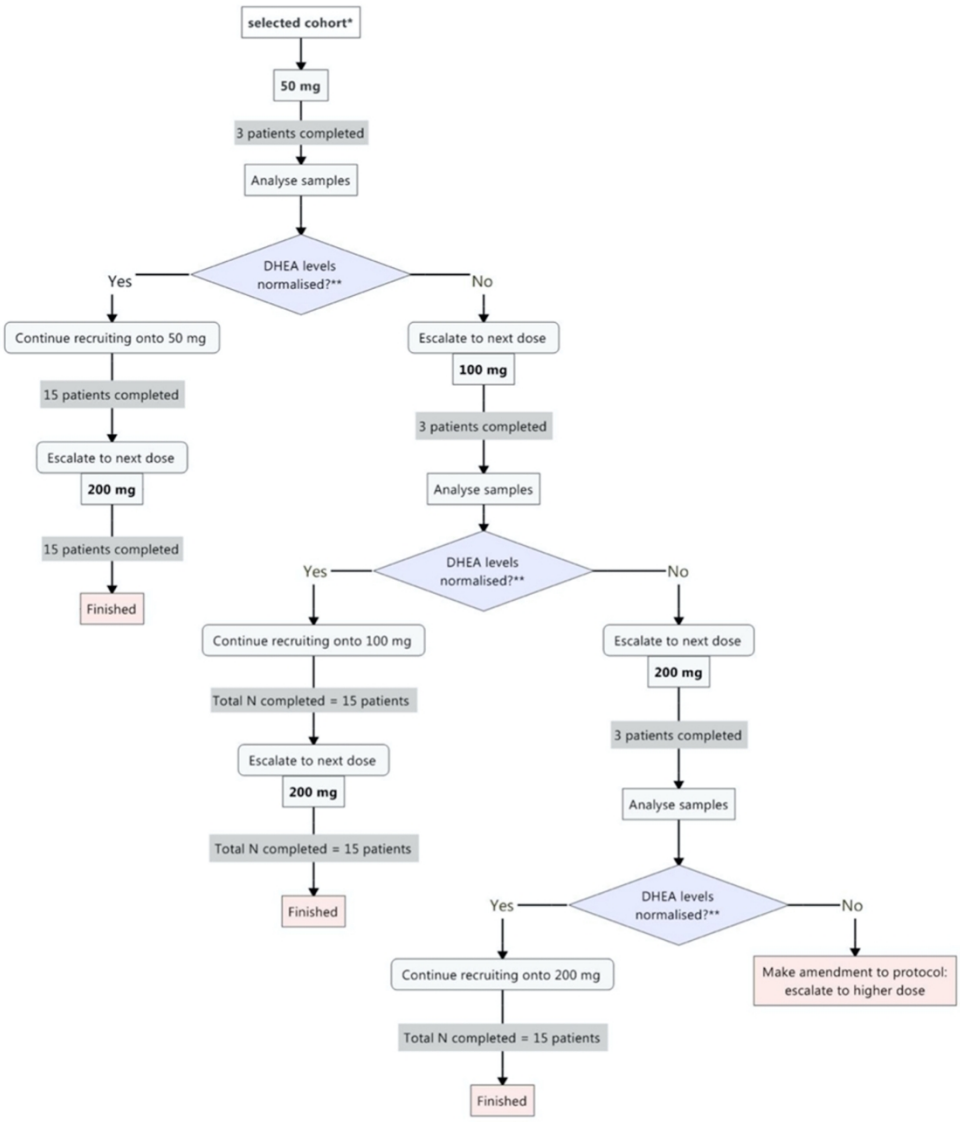

Figure 2: Indicative flowchart to explain dose escalation design of the ADaPT trial * Cohorts: oral-male trauma, sublingual-male trauma, oral-female trauma, sublingual-female trauma, oral-female hip fracture and sublingual-female hip fracture.

249x287mm (300 x 300 DPI)

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		Reporting Item	Page Number
Administrative information			
Title	#1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	1
Trial registration	#2a	Trial identifier and registry name. If not yet registered, name of intended registry	1
Trial registration: data set	#2b	All items from the World Health Organization Trial Registration Data Set	4 - ISRCTN
Protocol version	#3	Date and version identifier	10
Funding	#4	Sources and types of financial, material, and other support	23
Roles and responsibilities: contributorship	#5a	Names, affiliations, and roles of protocol contributors	23, 24

Roles and responsibilities: sponsor contact information	#5b	Name and contact information for the trial sponsor	24
Roles and responsibilities: sponsor and funder	#5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities	23
Roles and responsibilities: committees	#5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)	19
Introduction			
Background and rationale	#6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention	5
Background and rationale: choice of comparators	#6b	Explanation for choice of comparators	8
Objectives	#7	Specific objectives or hypotheses	9
Trial design	#8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, non-inferiority, exploratory)	9,10
Methods: Participants, interventions, and outcomes			
Study setting	#9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will	7, 8, 9. The study is single site

		be collected. Reference to where list of study sites can be obtained	
Eligibility criteria	#10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists)	11
Interventions: description	#11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	8, 13, Fig 2
Interventions: modifications	#11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving / worsening disease)	8, 13, 19
Interventions: adherance	#11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return; laboratory tests)	13, 19, dose-finding and recorded by nurses.
Interventions: concomitant care	#11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	Table 1
Outcomes	#12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended	16, 20, 21
Participant timeline	#13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure)	9, Fig 1.
Sample size	#14	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations	3

Recruitment	#15	Strategies for achieving adequate participant enrolment to reach target sample size	4,9,13,14
Methods: Assignment of interventions (for controlled trials)			
Allocation: sequence generation	#16a	Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions	13
Allocation concealment mechanism	#16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned	13
Allocation: implementation	#16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	13,Fig 1
Blinding (masking)	#17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	n/a
Blinding (masking): emergency unblinding	#17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	n/a
Methods: Data collection, management, and analysis			
Data collection plan	#18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests)	13, 17,18

		along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol	
Data collection plan: retention	#18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols	n/a – evaluable after just one dose.
Data management	#19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol	13, 19
Statistics: outcomes	#20a	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol	20,21
Statistics: additional analyses	#20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	n/a – already allocated into groups.
Statistics: analysis population and missing data	#20c	Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation)	21
Methods: Monitoring			
Data monitoring: formal committee	#21a	Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed	19
Data monitoring: interim analysis	#21b	Description of any interim analyses and stopping guidelines, including who will have access to these	9, 14, 19

		interim results and make the final decision to terminate the trial	
Harms	#22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct	22,13
Auditing	#23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	22
Ethics and dissemination			
Research ethics approval	#24	Plans for seeking research ethics committee / institutional review board (REC / IRB) approval	4
Protocol amendments	#25	Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC / IRBs, trial participants, trial registries, journals, regulators)	3, 15, 22
Consent or assent	#26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	10
Consent or assent: ancillary studies	#26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	n/a – dose-finding study.
Confidentiality	#27	How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial	13
Declaration of interests	#28	Financial and other competing interests for principal investigators for the overall trial and each study site	22
Data access	#29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators	23

Ancillary and post trial care	#30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation	Fig. 1. (follow-up two days post last dose).
Dissemination policy: trial results	#31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions	22
Dissemination policy: authorship	#31b	Authorship eligibility guidelines and any intended use of professional writers	22
Dissemination policy: reproducible research	#31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	23
Appendices			
Informed consent materials	#32	Model consent form and other related documentation given to participants and authorised surrogates	Due to nature of patient population, 3 ICF and 3 PIS are available.
Biological specimens	#33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable	n/a

BMJ Open

A prospective, phase II, single-centre, cross-sectional, randomised study investigating Dehydroepiandrosterone supplementation and its Profile in Trauma: ADaPT

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A prospective, phase II, single-centre, cross-sectional, randomised study investigating Dehydroepiandrosterone supplementation and its Profile in Trauma: ADaPT

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ABSTRACT

Introduction: The improvements in short-term outcome after severe trauma achieved through early resuscitation and acute care can be offset over the following weeks by an acute systemic inflammatory response with immuneparesis leading to infection, multi-organ dysfunction/failure (MOD/MOF) and death. Serum levels of the androgen precursor dehydroepiandrosterone (DHEA) and its sulphated ester DHEAS, steroids with immune-enhancing activity, are low after traumatic injury at a time when patients are catabolic and immunosuppressed. Addressing this deficit and restoring the DHEA(S) ratio to cortisol may provide a range of physiological benefits, including immune modulatory effects.

Objective: Our primary objective is to establish a dose suitable for DHEA supplementation in patients after acute trauma to raise circulating DHEA levels to at least 15 nmol/L. Secondary objectives are to assess if DHEA supplementation has any effect on neutrophil function, metabolic and cytokine profiles and which route of administration (oral vs sub-lingual) is more effective in restoring circulating levels of DHEA, DHEAS and downstream androgens.

Methods and analysis: ADaPT is a prospective, phase II, single-centre, cross-sectional, randomised study with a planned recruitment between April 2019 and July 2021 that will investigate DHEA supplementation and its effect on serum DHEA, DHEAS and downstream androgens in trauma. A maximum of 270 patients will receive sublingual or oral DHEA at 50, 100 or 200 mg daily over 3 days. Females aged ≥50 years with neck of femur fracture and male and female major trauma patients, aged 16-50 years with an injury severity score ≥ 16, will be recruited.

Ethics and dissemination: This protocol was approved by the West Midlands – Coventry and Warwickshire Research Ethics Committee (Reference 18/WM/0102) on 8th June 2018. Results

will be disseminated via peer-reviewed publications and presented at national and international conferences.

Trial registration: This trial is registered with the European Medicines Agency (EudraCT: 2016-004250-15) and ISRCTN (12961998). It has also been adopted on the National Institute of Health Research portfolio (CPMS ID:38158).

Trial Progression: The study recruited its first patient on 2nd April 2019 and held its first data monitoring committee on 8th November 2019. As of May 2020 there were 23 enrolled patients, with both male cohorts increasing to 100 mg. All female groups remain on 50 mg DHEA.

Article Summary

Strengths and limitations of this study

►► Identify the dose and route of administration needed to normalise DHEA levels in a cohort that are known to have low levels, both habitually and post-traumatic injury.

►► The rapid turnaround time from bedside to bench and bench to interim analysis will minimise inappropriate dosing and public money expenditure on ineffective doses.

►► This swift analysis will limit patient exposure to insufficient dosing and unnecessary specimen collection.

►► A study limitation could be its single-site design; however, this will facilitate collecting sensitive immunological samples.

Keywords: steroids, geriatric trauma, major trauma, endocrine, DHEA; DHEAS; immune function

Introduction

The improvements in short-term outcome after severe trauma achieved through early resuscitation and acute care are offset over the following weeks by an acute systemic inflammatory response with immuneparesis leading to infection, multi-organ dysfunction/failure (MOF) and death[1,2]. The combination of excessive pro- and anti-inflammatory responses impair the rehabilitation phase of trauma, including wound healing, physical and emotional recovery[3]. Upregulation of glucocorticoid biosynthesis promotes a catabolic state, lasting several weeks and associated with a significant reduction in muscle mass[4].

Analysis of gender differences in trauma has shown that women, particularly those under 30 years of age, have fewer infections and a better outcome from sepsis than men[5,6]. The protective effects of oestrogens on immune cells and organ function highlight the potential role of sex hormones in modulating trauma related immune dysfunction[7]. Cellular immunity is also influenced by hormone production, and members of our group have shown that the adrenal response to stress, specifically the ratio of cortisol to dehydroepiandrosterone sulphate (DHEAS), is linked to neutrophil bactericidal function, specifically superoxide production[8,9].

Dehydroepiandrosterone (DHEA) and its sulphate ester DHEAS are the most abundant steroids in the human circulation; DHEA is a precursor of sex hormones modulating several physiologic processes including metabolism, muscle protein synthesis and cardiovascular function[10,11]. DHEA is converted to active androgens in peripheral target cells including immune cells[12] and is also converted to DHEAS by the action of DHEA sulfotransferase (SULT2A1)[13]. We have shown that DHEA sulphation is down-regulated in acute inflammation systemic inflammatory response syndrome (SIRS) and sepsis [14]. Hazeldine et al reviewed the

numerous immune effects of DHEA [15] highlighting how DHEAS, but not DHEA, enhances neutrophil superoxide generation via a protein kinase C (PKC) mediated pathway, a vital immune response in fighting bacterial infections[16].

The roles of DHEA and DHEAS in severe injury are relatively unexplored. The majority of studies focus upon cortisol responses[17], whereas our data suggest that it is the cortisol: DHEAS ratio post-trauma that has a superior prognostic ability[18,19]. Although critical for survival, prolonged hypercortisolaemia is known to be detrimental in part due to its immunosuppressive effects[20]. This intra-adrenal shift causes decreased levels of circulating testosterone and oestrogen, resulting in rapid lean body mass loss[21], in addition to increased susceptibility to infection and sepsis[22]. Correcting the cortisol: DHEA or cortisol: DHEAS ratio via the administration of DHEA has yet to be undertaken in traumatically-injured patients despite DHEA and DHEAS being below the reference ranges for 6 weeks and 6 months post-injury respectively[4].

DHEA is subject to first-pass metabolism in healthy individuals, which in turn may lead to a non-physiological metabolism after an oral dose[23]. Bypassing first-pass metabolism using a sublingual or buccal preparation[24] should improve the bioavailability of DHEA [25,26]. Previous studies in the healthy older people have shown that supplementation with 50 mg DHEA orally once daily can restore both serum DHEA and DHEAS levels to that of men and women in the third decade of life[23,27]. Moreover, the current literature suggests that DHEA supplementation may not only be beneficial for immune function but extend to bone remodelling, muscle composition, psychological and neurological improvements[28].

This study will seek to estimate the optimal dose and route of administration in trauma patients of DHEA to increase serum levels of DHEA and DHEAS to those seen in healthy adults. We also aim to find the optimal dose to enhance immune function, as demonstrated through

changes in neutrophil phagocytosis and ROS production. The pilot data generated from the study is necessary to develop the protocol for a randomised controlled trial that will determine whether DHEA supplementation may improve outcomes in injured patients.

Rationale

Justification of the patient population

Although improvements in short-term outcomes from traumatic injury via aggressive early resuscitation and acute care, over 5 million people worldwide die each year from serious injury[29]. With improved pre-hospital medicine mitigating the immediate threats to life, it is the following weeks after traumatic injury, that has seen the dysregulated SIRS in susceptible patients. The SIRS response is accompanied by a compensatory anti-inflammatory response (CARS)[30] to restore homeostasis. SIRS and CARS may progress to the persistent inflammation, immunosuppression and catabolism syndrome (PICS)[31,32]. PICS further compounds the insult from the initial injury and results in an increased risk of infection, MOF and late deaths[1,2]. Therefore, strategies to mitigate these detrimental outcomes for patients are needed in the short, medium and long-term care of trauma patients.

The young trauma cohort will be recruited alongside a cohort of older (≥50 years old) female patients who have sustained a hip fracture at the neck of femur (NOF). According to the National Hip Fracture Database (2018), the NOF burden upon the UK economy is estimated to be around £1bn per annum[33]. This considerable cost is set to rise as the population ages. By the age of 40, decreasing serum levels of DHEA and DHEAS are observed in both sexes[34,35]; a phenomenon sometimes referred to as “adrenopause”. Circulating levels of DHEA and DHEAS are lower in women than in men; however, in post-menopausal women, adrenal

androgens are a source of almost all active oestrogens[36]. To the best of our knowledge, there has been no traumatic injury or NOF interventional studies supplementing DHEA or DHEAS, despite its therapeutic potential in the immediate to longer-term care of the young and aged trauma patient. In this pilot study, we will recruit a female NOF cohort as well as a young adult trauma cohort with both male and female patients presenting at the MTC and Intensive Care Unit (ICU) at QEHB.

Choice of treatment

The DHEA doses chosen in this study (50 mg, 100 mg and 200 mg) were selected based upon *in vivo* studies that demonstrated these doses to be both safe and effective at raising the levels of DHEA to that of healthy young adult levels[37]. DHEA is at its highest in the third decade of life. After which there is a steady decrease over the life course in both males and females. These doses have also previously been used in adrenal insufficiency[38], older people[39], and young males[40] and females[24], causing transient hyperandrogenism with acne being the most frequently reported side effect[41,42]. DHEA is activated to downstream androgens by stepwise conversion catalysed by several steroidogenic enzymes. However, we do not know whether the expression and activity of these enzymes are affected by major trauma and inflammation. There is evidence that inflammation and trauma affect DHEA sulfation[4,14] and may shift the conversion of DHEA to a higher rate of androgen activation.

Trauma and inflammation can impact adversely on gut absorption [43] and hepatic first-pass metabolism [44]. Therefore, we have chosen to administer DHEA as a sublingual preparation. Sub-lingual administration is commonly used by nursing staff in the ICU context, especially in those patients who have contraindications to oral administration or by patients wishing to self-administer [45].

OBJECTIVES

Primary Objective

The primary objective of this study is to establish the daily dose of DHEA that restores serum DHEA levels to at least 15 nmol/L, i.e. the mid healthy adult reference range, in trauma and hip fracture patients after 3 days of supplementation.

Secondary Objective

Secondary objectives include observing the effect of single and multiple DHEA doses on circulating DHEA, DHEAS and downstream androgens. Additionally, we will investigate whether route of DHEA administration (oral vs sublingual) modulates the profile of circulating DHEA. This will be determined by assessing the efficacy of each route via statistical modelling. The immune response to the DHEA supplementation will be assessed as a marker of potential clinical relevance.

METHODS

Trial Design

ADaPT is a prospective, randomised, open-label, trial conducted in male and female adult trauma patients and older females who have suffered a fracture of the neck of the femur. This is a single-site study with patients recruited from Queen Elizabeth Hospital, Birmingham, UK (QEHB). Three doses of DHEA will be investigated in this trial: 50, 100, 200 mg, each administered once daily for 3 days via either oral or sublingual tablets. The trial has an adaptive design in order to answer both the primary and secondary objectives, with regular interim analysis to minimise the investigation of inactive doses. The trial consists of two components. The first component of the trial is to determine the dose of DHEA needed to sufficiently raise serum DHEA levels to at least 15 nmol/L after 3 days of DHEA administration.

Based on previous work, 15 nmol/L has been selected as this is the lower acceptable level of DHEA observed in healthy young adults. Our recent analysis of the steroid response to trauma has shown that DHEA levels were very low and often undetectable for several weeks after trauma [4]. The second component of the trial is to investigate if DHEA will enhance neutrophil function. The study was approved by The Medicines and Healthcare products Regulatory Agency (MHRA) for the use of DHEA as an investigational medicinal product. Ethical approval was obtained from the West Midlands Research Ethics Committee (Reference 18/WM/0102). The trial will be conducted in accordance with the Declaration of Helsinki (World Medical Association 2008). **Figure 1** summarises the patient pathway of the trial. The protocol (version 5.0, 28th June 2019) has been prepared in accordance with the SPIRIT guidelines [46].

Patient selection

A maximum of 270 patients will be enrolled across three patient groups (young male trauma, young female trauma and female hip fracture). These trauma patient groups have been selected due to the immuneparesis effects caused by the acute systemic inflammatory response that follows severe trauma. The hip fracture group was selected as they have low serum DHEA and DHEAS due to adrenopause, and there are several papers showing an association between HPA axis activity measures and outcomes in these patients[9,19,47,48]. Patients admitted to QEHB will all be pre-screened and assessed for eligibility. Patients will be screened between 07:00 – 20:00, 7 days a week. Potential participants will have an assessment of their medical history and current trauma injury and the eligible patients will be recruited. The eligibility criteria have been split into trauma patients and hip fracture patients (**Table 1**). The study will not exclude NOF patients with dementia where supplementation is

currently being tested in the prevention and treatment of age-related cognitive impairment without deleterious effects [49].

Table 1 Patient inclusion and exclusion criteria

Trauma patients inclusion criteria
- Aged 16 - 50 years of age
- Severely injured trauma patient (Injury severity score (ISS) ≥16 and ≤50)
- Admitted to University Hospital Birmingham within 6 days of trauma
- Anticipated to be an inpatient for the 12-day trial period
Trauma patients exclusion criteria
- Ages <16 or >51
- ISS <16 or >50
- Isolated brain injury
- Unlikely to survive the study period
- Known hormone sensitive malignancy
- Known Prostatic hypertrophy (M)
- Female patients taking HRT medication or oral contraceptives
- Intake of any drugs that interfere with adrenal function in the last 3 months:
Increased metabolism of glucocorticoids • corticosteroids
Impaired glucocorticoid action Peripheral glucocorticoid insensitivity • Glucocorticoid receptor antagonist—mifepristone. • Suppression of glucocorticoid-induced gene transcription—chlorpromazine, imipramine.
Inhibition of steroidogenic enzymes involved in cortisol production • Inhibition of mitochondrial (type 1) cytochrome P450 enzymes (CYP11A1, CYP11B1, CYP11B12)—ketoconazole, fluconazole, itraconazole, etomidate, metyrapone, aminoglutethimide. • Inhibition of 3β-HSD2—trilostane.
- Pre-existing liver impairment or chronic liver failure
- Previous or current malignancy or invasive cancer diagnosed within the past 3 years except for adequately treated basal cell and squamous cell carcinoma of the skin and in situ carcinoma of the uterine cervix
- Pregnant and/or breast-feeding females (women of childbearing potential to complete serum pregnancy test)
- Known hypersensitivity to the active substance or excipient
- Known thromboembolic events in the last 12 months and any predisposition to thrombosis e.g. factor V leiden
Hip fracture patients inclusion criteria
- Aged 50 years and older
- Female
- Neck of femur fracture
- Admitted to University Hospital Birmingham within 6 days of fracture

- Anticipated to be an inpatient for the 12 day trial period
Hip Fracture patients exclusion criteria
- <50 years old
- Unlikely to survive the study period
- Previous or known hormone sensitive malignancy
- Intake of any drugs that interfere with adrenal function in the last 3 months: **Increased metabolism of glucocorticoids** • Concomitant use reduces corticosteroid concentrations **Impaired glucocorticoid action** Peripheral glucocorticoid insensitivity • Glucocorticoid receptor antagonist—mifepristone. • Suppression of glucocorticoid-induced gene transcription—chlorpromazine, imipramine. **Inhibition of steroidogenic enzymes involved in cortisol production** • Inhibition of mitochondrial (type 1) cytochrome P450 enzymes (CYP11A1, CYP11B1, CYP11B12)—ketoconazole, fluconazole, itraconazole, etomidate, metyrapone, aminoglutethimide. • Inhibition of 3β-HSD2—trilostane.
- Pre-existing liver impairment or chronic liver failure
- Previous or current malignancy or invasive cancer diagnosed within the past 3 years except for adequately treated basal cell and squamous cell carcinoma of the skin and in situ carcinoma of the uterine cervix - Pregnant and/or breastfeeding (women of childbearing potential to complete serum pregnancy test)
- Known hypersensitivity to the active substance or excipient
- Females on Hormone Replacement Therapy medication
- Known thromboembolic events in the last 12 months and any predisposition to thrombosis e.g. factor V leiden

Randomisation

Patients who meet the eligibility criteria and provide consent are randomised to receive DHEA via either oral tablets or sublingual tablets once daily for 3 days using a 1:1 randomisation ratio. With three populations of patients (male-trauma, female-trauma and female-hip fracture) and two routes of administration, there will be 6 groups in total. Randomisation will take place using a secure web-based tool. Nursing and medical staff will use the Clinical RESearch Tool (CREST), developed at University Hospitals Birmingham Foundation Trust (UHBFT), to randomly assign patients and provide an anonymised electronic case report form,

for trial management, data collection and adverse event reporting. The prevailing dose of DHEA (50, 100 or 200 mg) for administration in a group will be adapted in response to sequential analysis of interim outcomes. Each group will have its own dose. Blinding is not possible as the difference in DHEA delivery method is evident to both the clinician and the participant. If a contraindication to oral or the sublingual route present prior to commencing the study intervention, forced randomisation will occur.

Study Intervention

The supplementation of DHEA will commence in the second week after injury, which has previously been shown to be a time when trauma patients become maximally unwell as a result of sepsis and multiple organ failure[50]. Additionally, this time point has been selected to optimise patient recruitment from both the NOF cohort and trauma patients (median stay 18 days, and 13 days respectively) both DHEA and DHEAS levels post-injury [51]. Recruiting an in-hospital cohort provides an opportunity to monitor patients and assess the impact that this class C controlled drug has upon the serial steroid profile and immune function during a period of vulnerability over 3 days of administration.

Participant timeline

The trial intervention will occur over three days, during the first 12 days while inpatients at QEHB. Three doses of DHEA will be investigated in all patient, and treatment will occur on day 8, 9 and 10 only. All cohorts will begin the study on 50 mg, and the dose administered to the next eligible patient to be included within a cohort will be escalated when interim analysis

determines if the primary objective has been reached. At no point will any patient escalate once they have been allocated a dose of DHEA.

Dose escalation

Dose escalation for the DHEA restoration part of the trial is dependent upon the serum DHEA levels. A dose that restores serum DHEA to ≥15 nmol/L (referred to as 'normalise DHEA') is sought in at least 13-out-of-15 patients or approximately 85% of patients. The decision to escalate dose in a cohort will be driven in the main by the outcomes of the patients in that cohort and escalation will occur independently for each cohort. However, valuable additional information will be garnered from the outcomes of patients in other cohorts. Flexible information sharing across related groups will be achieved using hierarchical regression models. The requirement for 13-out-of-15 patients to normalise in the final effective dose is based on what was judged to be clinically important, and thus would warrant investigation of the chosen dose of DHEA in a confirmatory phase III trial to ascertain superiority of outcomes compared to standard of care.

To minimise the investigation of inactive doses, we propose an analysis of each dose-level in each cohort after n = 3-5 evaluable patients have been assessed. It will be taken as evidence that the normalisation rate is too low and that the dose should be increased if: any patient fails to normalise (where n=3); or more than one patient fails to normalise (where n=4 or 5). This rule-based analysis will provide an early opportunity to escalate in search of a more promising dose when a probabilistic analysis will likely be under-informed due to the small sample size. If the requisite number normalise, recruitment will continue in that cohort at that dose-level up to a maximum of n=15.

Once an effective dose has been established to normalise DHEA levels within a group and at least n = 15 have completed it, the DHEA will be escalated to the next dose to satisfy the

second component of the trial; to determine whether further increases in DHEA supplementation will enhance immune function. The immune response component will focus on neutrophil phagocytosis and ROS production which will involve fewer research samples. N = 15 patients (within a cohort) will complete the immune response part of the trial at each subsequent dose escalation. If 50 mg is established to be sufficient to normalise DHEA the dose will be escalated twice to investigate whether 100 mg or 200 mg is optimal for increasing the immune response. Both 100mg and 200mg have safely been used in previous studies[52,53], but this has not been addressed in the context of trauma. Each group will be escalated independently of each other (**figure 2**).

Patient and public involvement (PPI)

Before the beginning of the study, patients and lay members of the 1000 elders group at the University of Birmingham were invited to group PPI sessions held by the Surgical Reconstruction and Microbiology Reconstruction Centre (SRMRC) at QEHB. During these interactive group sessions, discussions were undertaken to highlight the work that was planned to be undertaken to address previously highlighted problems in their recovery from traumatic injury. Members of the group informally looked at, developed and passed comment upon study design and patient paperwork- contemporaneous records were generated. Upon entry and active participation with the ADaPT study, patients were asked if they would like to become members of the PPI group and assist in the ongoing evaluation and future dissemination of the project. The participants will be asked to participate in a grant

application should the results of this study be warranting a more extensive phase 3 multicentre trial.

Primary and secondary outcomes

The primary outcome for the study is serum DHEA after 3 days of DHEA supplementation. Previous research into DHEA levels and DHEA supplementation use DHEAS as the primary endpoint for determining whether the supplementation has been effective at raising levels. However, DHEAS levels do not act as a proxy marker for DHEA levels in the trauma population[51]. Utilising liquid chromatography-tandem mass spectrometry (LC-MS/MS) we have shown that DHEA and DHEAS both behave differently after trauma injury[54]. Animal models of trauma have demonstrated improvements in hyperglycaemia[55], decreased mortality after trauma-induced haemorrhage[56], neurogenesis[57] and wound reperfusion[58]. Human studies including a recent meta-analysis suggested that DHEA supplementation may be beneficial in increasing bone mineral density (BMD)[59] in women, increasing muscle strength[60], improving mood in those with moderate depression[61] and adrenal insufficiency [38]. These potential restorative immunological, physiological and psychological benefits seen in animals and human studies can only be investigated once the appropriate dose of DHEA to restore normal levels and the most suitable administration route has been identified. We know that supplementation of DHEA in healthy subjects via oral administration will result in significant first-pass metabolism and, thus, more extensive conversion of DHEA to DHEAS than is observed via, e.g. transdermal administration [62] which is why we plan to compare oral vs sublingual DHEA administration.

One potential instantaneous benefit to trauma patients, which may be observed systemically after a dose of DHEA, is a positive effect upon the bactericidal function of neutrophils[16] by enhancing reactive oxygen species (ROS) generation via activation of NADPH oxidase[63].

Neutrophil function will therefore be investigated as a secondary outcome, with limited expectations on the results of the pilot data, given that DHEA will only be administered for 3 days.

Steroid Analysis

DHEA and downstream androgens will be quantified using a validated liquid chromatography-tandem mass spectrometry (LC-MS/MS) multi-steroid profiling method carried out on a Waters Xevo-XS, with acquity uPLC, using a water/methanol (0.1% formic acid) gradient system and a HSS T3, 1.8 µm, 1.2x50 mm column. Steroids are extracted via liquid-liquid extraction using tert-butyl methyl ether (MTBE), following the addition of an internal standard and protein precipitation using acetonitrile. The MTBE layer containing steroids was transferred and evaporated under nitrogen then reconstituted in 125 µL of 50/50 methanol/water before analysis. Steroids will be quantified against a calibration series ranging from 0.05 to 250 ng/mL[64–68].

DHEAS was measured separately. 20 µL of serum was spiked with internal standard, followed by 100 µL of acetonitrile and 20 µL of ZnSO4. The samples were then centrifuged and 100 µL of the solution was transferred to a new vial, dried and reconstituted in 200 µL of methanol/water prior to liquid chromatography tandem mass spectrometry analysis as described by Chadwick et al[69]. DHEAS will be quantified against a calibration series ranging from 250 to 8000 ng/mL.

Neutrophil Function

Trauma initiates a "stress response" through the endocrine, metabolic and inflammatory systems. The primary endocrine response is to produce catecholamines and corticosteroids, raising the immune response and mobilisation of neutrophils[70]. Neutrophil function will be analysed through the validated PhagoBURST™ and PhagoTest™ kits (Glycotope Biotechnology GmbH, Germany) to assess superoxide generation and phagocytosis, respectively. This gives a pilot opportunity to test whether DHEA supplementation improves the immune response and in turn, has the potential to protect against infection. However, benefits might only be detectable after a period of DHEA supplementation that is substantially longer than three days.

Pro and anti-inflammatory Cytokines

Prolonged CARS may leave the recovering patient susceptible to increased risk of late infection[71]. The cytokine storm of IL-6 and IL-10 have demonstrated a strong association with the severity of injury and mortality [72], and less so sepsis[73]. These post-injury cytokines are also known to affect the peripheral target tissues that are involved in steroid metabolism[74]. The post-injury pro and anti-inflammatory cytokines assessed have been selected based on previous work from our group[75].

Tolerance – gastric residual volume

Swallowing difficulties, facial injuries or a non-functioning gut may prohibit sublingual or oral administration and compliance to the study protocol. Therefore, an adaptable study design is needed to generate pilot data. By monitoring gastric residual volumes (GRV), a surrogate marker of gastrointestinal motility[76], we will regard a GRV persistently exceeding 250ml as intolerable.

Treatment Compliance and Evaluability

To meet study compliance and be considered evaluable, the following must be satisfied:

- Patients must be sufficiently dosed on at least one day of DHEA administration.
- If a patient fails to consume the intended IMP, or vomits within 1 hour of consuming the IMP, this dosing will be classed as *insufficient*.

Data Monitoring Committee

Data analyses will be supplied in confidence to an independent Data Monitoring Committee (DMC), which will be asked to advise on whether the accumulated data from the trial, together with the results from other relevant research, justify the continuing recruitment of further patients. The DMC will operate under a trial-specific charter based upon the template created by the Damocles Group.

Results will be provided to the DMC and discussed via teleconference at a minimum. In consultation with the trial statistician, the DMC will meet when any cohort undergoes a dose escalation decision. The DMC will advise on dose escalation based on the rules as described previously. Additional meetings may be called if recruitment is much faster than anticipated and the DMC may, at their discretion, request to meet more frequently or continue to meet following completion of recruitment. An emergency meeting may also be convened if a safety issue is identified. The DMC will report directly to the Trial Management Group. The DMC may consider recommending the discontinuation of the trial if the recruitment rate or data quality is unacceptable or if any issues are identified, which may compromise patient safety. The trial would also stop early if the interim analyses showed differences between treatments that were deemed to be convincing to the clinical community. Data monitoring members have undertaken their initial review of the first sixteen patients on the 8th November 2019. The

outcome of this DMC required all-female cohorts to continue 50 mg of DHEA (both the sublingual and oral), with both male cohorts increasing to 100 mg.

Statistical Analysis

Sample Size

The maximum sample size will be 270 (six groups of 15 participants being administered three different dose-levels). However, we predict realised sample size to be smaller as there are likely to be early opportunities to escalate dose-level within a group. Following consultation with a trial statistician n = 15 was chosen to provide a modest amount of information on the primary outcome at each dose in each group while allowing recruitment to be completed in a reasonable amount of time. Statistical power calculation has not been performed as ADaPT is a dose escalation trial and we are not applying a null hypothesis significance testing approach.

Primary outcome

Serum DHEA concentrations will be summarised as means and standard deviations (or medians and inter-quartile ranges, if non-normal) at each time-point and dose in each cohort, where a cohort is the three-way combination of patient cohort, dose and administration route. The observed rate of DHEA normalisation will be reported for each cohort with confidence intervals calculated using Wilson's method. The cohorts sample sizes are small, so these cohort-specific analyses are likely to be relatively imprecise. However, the total sample size is large for the trial phase, and there is much information in the cohort structure that will likely be pertinent to understanding the outcomes. Supplementary analyses to support dose decisions will be provided using hierarchical regression models that analyse outcomes from

all cohorts and doses together while reflecting cohort memberships. These models are explained below.

Modelling serum DHEA concentrations and DHEA-normalisation probability

We propose hierarchical Bayesian models to analyse serum DHEA outcomes in all cohorts simultaneously. An intercept will be included to estimate the mean population-level response common to all cohorts plus further terms to reflect effects for dose, patient type, and administration method. Further population-level terms (also called fixed effects) will be considered, including baseline DHEA level and age. Interactions will be considered, as appropriate. Group-level terms (also called random effects) to reflect cohort and patient heterogeneity will be considered.

Modestly informative or regularising priors will be used that anticipate little or no effect (i.e. have a mean close to or equal to zero) but restrict the range of parameter values to those that are feasible (i.e. do not place undue or unrealistic probability mass in wide distribution tails). Such priors can be considered informative of scale but not location in that they do not anticipate effects but rule out infeasible values. They are effective at dissuading models from over-fitting and aiding convergence in the posterior sampler when there are many parameters. Information criteria (e.g. WAIC or LOOIC) will be used to find parsimonious but informative models.

It is anticipated that: the probability of DHEA normalisation will be modelled using a generalised linear model with logit link function, and post-baseline DHEA will be modelled using a generalised linear model with identity or log (for positive data) link functions.

Interim Analysis

There will be an interim analysis presented when any cohort undergoes a dose-escalation decision, as previously described. The particular objective of this analysis will be to assess if the rate of DHEA normalisation is too low and whether there is evidence that motivates investigating a higher dose in that cohort. The primary and supporting analyses of the primary outcome will be presented, as described above.

Ethics and Dissemination

This protocol has been approved by a Research Ethics Committee (Reference 18/WM/0102) on 8th June 2018. Results will be disseminated via peer-reviewed publications and presented at national and international conferences. This will be coordinated with members of the research team, both past and present. The study investigator is responsible for communicating important protocol modifications to relevant parties.

Trial Progression

The study recruited its first patient on 2nd April 2019 and held its first data monitoring committee on 8th November 2019. Currently, there are 23 evaluable patients, with both male cohorts increasing to 100 mg. All female groups remain on 50 mg DHEA. The dose escalation also coincided with the first sponsor audit of ADaPT. Site audits will occur at times of escalation and interim analysis until the study is completed.

Figure Legends

Figure 1: A trial flowchart describing the patient journey in through the ADaPT study.

* Due to the nature of the injury, informed consent can be sought from a professional legal representative or personal legal representative if the patient does not have capacity. Consent from the patient will be obtained at the earliest opportunity by research team members.

**24hr bloods and consent will only be collected within 24hrs of injury. The omission of this sample collection does not render a patient unevaluable.

Figure 1: Indicative flowchart to explain dose escalation design of the ADaPT trial

* Cohorts: oral-male trauma, sublingual-male trauma, oral-female trauma, sublingual-female trauma, oral-female hip fracture and sublingual-female hip fracture.

Declarations

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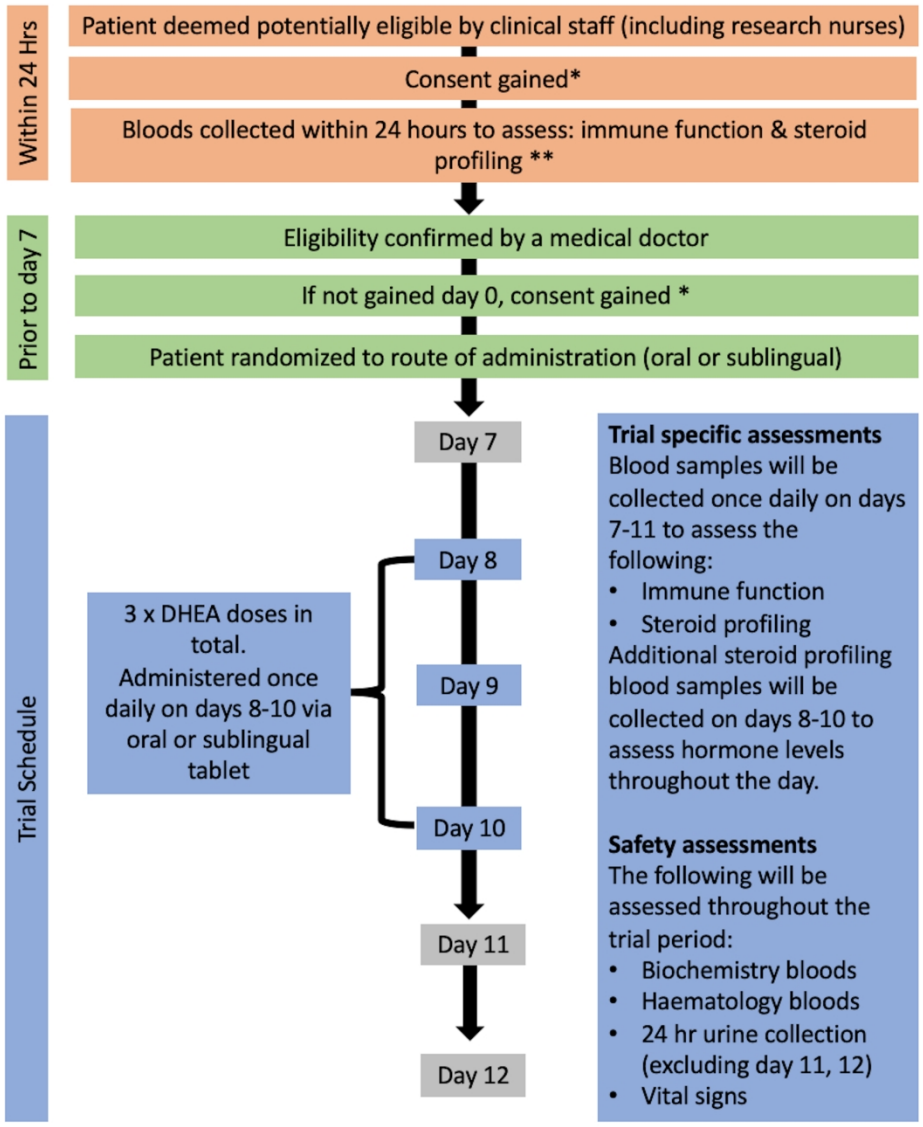

Figure 1: A trial flowchart describing the patient journey in through the ADaPT study.
* Due to the nature of the injury, informed consent can be sought from a professional legal representative or personal legal representative if the patient does not have capacity. Consent from the patient will be obtained at the earliest opportunity by research team members.
**24hr bloods and consent will only be collected within 24hrs of injury. The omission of this sample collection does not render a patient unevaluable.

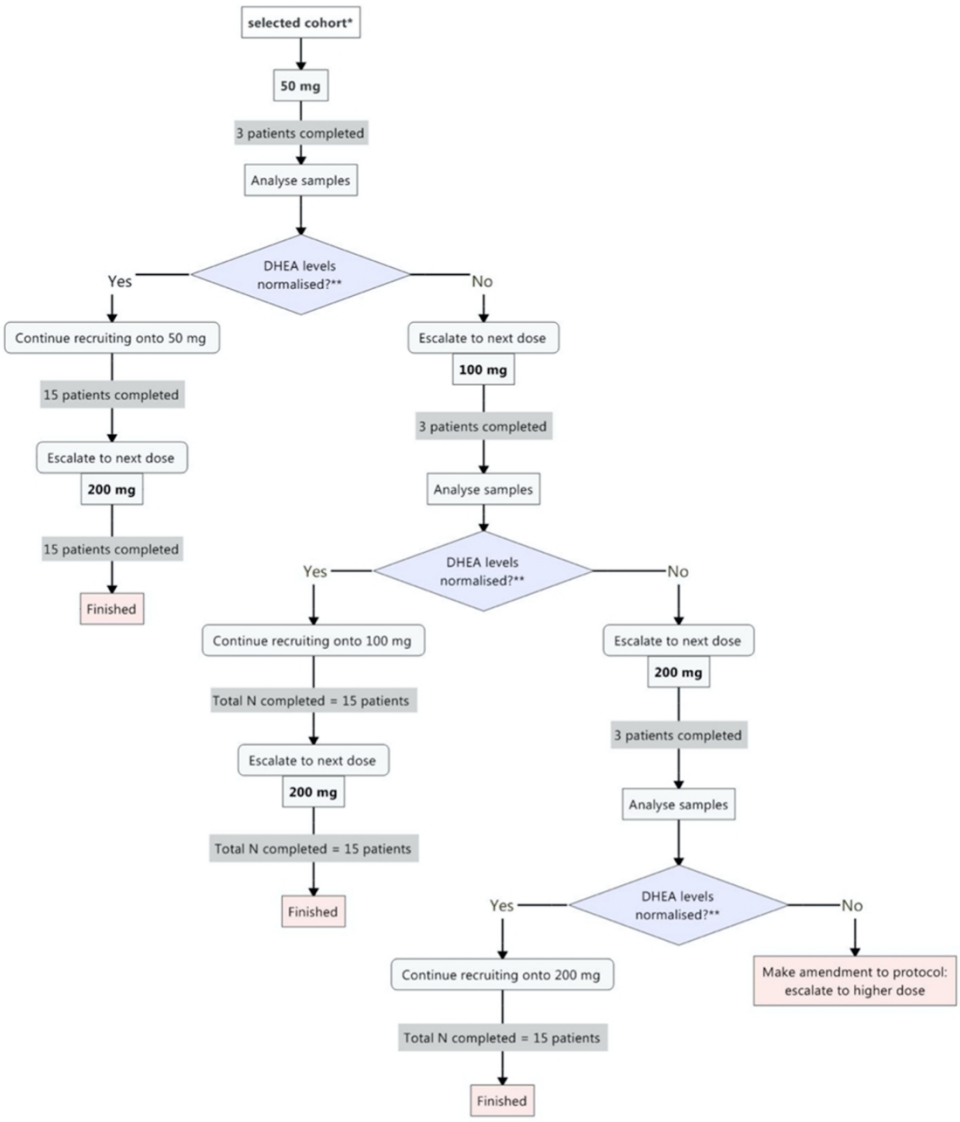

Figure 2: Indicative flowchart to explain dose escalation design of the ADaPT trial * Cohorts: oral-male trauma, sublingual-male trauma, oral-female trauma, sublingual-female trauma, oral-female hip fracture and sublingual-female hip fracture.

249x287mm (600 x 600 DPI)

Reporting checklist for protocol of a clinical trial.

Based on the SPIRIT guidelines.

Instructions to authors

Complete this checklist by entering the page numbers from your manuscript where readers will find each of the items listed below.

Your article may not currently address all the items on the checklist. Please modify your text to include the missing information. If you are certain that an item does not apply, please write "n/a" and provide a short explanation.

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		Reporting Item	Page Number
Administrative information			
Title	#1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	1
Trial registration	#2a	Trial identifier and registry name. If not yet registered, name of intended registry	1
Trial registration: data set	#2b	All items from the World Health Organization Trial Registration Data Set	4 - ISRCTN
Protocol version	#3	Date and version identifier	10
Funding	#4	Sources and types of financial, material, and other support	23
Roles and responsibilities: contributorship	#5a	Names, affiliations, and roles of protocol contributors	23, 24

Roles and responsibilities: sponsor contact information	#5b	Name and contact information for the trial sponsor	24
Roles and responsibilities: sponsor and funder	#5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities	23
Roles and responsibilities: committees	#5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)	19
Introduction			
Background and rationale	#6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention	5
Background and rationale: choice of comparators	#6b	Explanation for choice of comparators	8
Objectives	#7	Specific objectives or hypotheses	9
Trial design	#8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, non-inferiority, exploratory)	9,10
Methods: Participants, interventions, and outcomes			
Study setting	#9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will	7, 8, 9. The study is single site

		be collected. Reference to where list of study sites can be obtained	
Eligibility criteria	#10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists)	11
Interventions: description	#11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	8, 13, Fig 2
Interventions: modifications	#11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving / worsening disease)	8, 13, 19
Interventions: adherance	#11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return; laboratory tests)	13, 19, dose-finding and recorded by nurses.
Interventions: concomitant care	#11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	Table 1
Outcomes	#12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended	16, 20, 21
Participant timeline	#13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure)	9, Fig 1.
Sample size	#14	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations	3

Recruitment	#15	Strategies for achieving adequate participant enrolment to reach target sample size	4,9,13,14
Methods: Assignment of interventions (for controlled trials)			
Allocation: sequence generation	#16a	Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions	13
Allocation concealment mechanism	#16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned	13
Allocation: implementation	#16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	13,Fig 1
Blinding (masking)	#17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	n/a
Blinding (masking): emergency unblinding	#17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	n/a
Methods: Data collection, management, and analysis			
Data collection plan	#18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests)	13, 17,18

		along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol	
Data collection plan: retention	#18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols	n/a – evaluable after just one dose.
Data management	#19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol	13, 19
Statistics: outcomes	#20a	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol	20,21
Statistics: additional analyses	#20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	n/a – already allocated into groups.
Statistics: analysis population and missing data	#20c	Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation)	21
Methods: Monitoring			
Data monitoring: formal committee	#21a	Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed	19
Data monitoring: interim analysis	#21b	Description of any interim analyses and stopping guidelines, including who will have access to these	9, 14, 19

		interim results and make the final decision to terminate the trial	
Harms	#22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct	22,13
Auditing	#23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	22
Ethics and dissemination			
Research ethics approval	#24	Plans for seeking research ethics committee / institutional review board (REC / IRB) approval	4
Protocol amendments	#25	Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC / IRBs, trial participants, trial registries, journals, regulators)	3, 15, 22
Consent or assent	#26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	10
Consent or assent: ancillary studies	#26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	n/a – dose-finding study.
Confidentiality	#27	How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial	13
Declaration of interests	#28	Financial and other competing interests for principal investigators for the overall trial and each study site	22
Data access	#29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators	23

Ancillary and post trial care	#30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation	Fig. 1. (follow-up two days post last dose).
Dissemination policy: trial results	#31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions	22
Dissemination policy: authorship	#31b	Authorship eligibility guidelines and any intended use of professional writers	22
Dissemination policy: reproducible research	#31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	23
Appendices			
Informed consent materials	#32	Model consent form and other related documentation given to participants and authorised surrogates	Due to nature of patient population, 3 ICF and 3 PIS are available.
Biological specimens	#33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable	n/a